DIFFERENTIAL INACTIVATION OF ADENOSINE BY HUMAN AND CANINE BLOOD

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Received 26 March 1969

1. Introduction

According to Berne's hypothesis on the metabolic regulation of coronary blood flow, adenosine is the main vasoactive substance released from the myocardial cell during hypoxia [1]. The dilatory effect of exogenous adenosine on the coronary vessels is short-lived, due to its rapid inactivation in the blood and/or tissues. Using a new and fully automatic colorimetric method for the continuous determination of adenosine [2], we have found marked qualitative and quantitative differences in the rate of disappearance of adenosine from human and canine blood. The present paper reports on these differences, and shows that although in human blood adenosine is mainly taken up by the erythrocytes, in canine blood it is mainly taken up by the platelets.

2. Materials and methods

Approximately 8 ml of blood from healthy male human volunteers or from male beagle dogs was collected by free flow from a venipuncture into a plastic tube containing 2 ml of ACD (acid-citrate-dextrose: USP XVI, 662). All manipulations were performed using plastic syringes or tubes, and care was taken to avoid haemolysis.

2.1. *Preparation of total blood samples*

0.1 ml of human blood or 2 ml of canine blood was diluted to 8 ml with isotonic phosphate buffer (pH 7.4).

2.2. *Preparation of erythrocyte samples*

Total blood samples were centrifuged for 15 min at 350 *g* to separate the erythrocytes and the platelet-rich plasma. The sedimented red cells were then diluted with physiological saline at pH 7.4, and approximately 8 ml was layered onto 25% Ficoll (Pharmacia, Uppsala) in saline. The erythrocytes were separated from the residual platelets either by allowing the mixture to stand for several hours or by centrifuging at low speed. The erythrocytes thus obtained were washed twice with saline, taken up in 3 ml of saline, and aliquots of 0.1 ml (human) or 1 ml (canine) of the suspension were diluted to 8 ml with isotonic phosphate buffer.

2.3. *Preparation of plasma samples*

The platelet-rich plasma was centrifuged for 25 min at 750 *g* to sediment most of the platelets, and a further 20 min at 2500 *g* to remove the remainder: 2 ml of the supernatant was then added to 6 ml of phosphate buffer.

2.4. *Preparation of platelet samples*

The platelet sediment was washed 3 times with physiological saline by sucking up and down in a syringe and centrifuging. Aliquots of 1 ml (human) or 0.5 ml (canine) of a suspension of the residue in 3 ml of saline were diluted to 8 ml.

2.5. *Preparation of lysates of total blood, erythrocytes and platelets*

Portions of the total blood, washed erythrocytes or platelets were diluted with water (1:4, v/v) and

sonicated for 2 X 1 min in ice-water using a Kerry Ultrasonicator at maximum power. For the human samples, aliquots comprising 0.5 ml of total blood lysate, 0.5 ml of erythrocyte lysate or 2 ml of platelet lysate were diluted to 8 ml with isotonic phosphate buffer. For the dog samples, 0.2 ml, 2 ml and 0.4 ml aliquots of the respective lysates were diluted to 8 ml.

Each 8 ml fraction in turn was incubated at 37°C, agitated with a slow speed magnetic stirrer and taken up continuously for analysis at a rate of 0.23 ml/min. After exactly 5 min, to permit baseline establishment, 0.787 μmoles of adenosine (= 300 μl of a solution containing 7 mg of adenosine/10 ml of phosphate buffer) was added to the incubation mixture, to give a final adenosine concentration of 110 μM. The amount of adenosine in the mixture was followed continuously, using the technique described in an earlier publication [2].

Erythrocytes, leucocytes and platelets were counted using a Fisher Autocytometer, except for the residual platelets in the erythrocyte suspension which were counted by direct microscopic examination.

3. Calculation procedure

The calculation procedure is illustrated in fig. 1. The heavy line ABC represents the (hypothetical) original trace; AB is the baseline and B the point at which 0.787 μmoles of adenosine were added to the incubation mixture. The narrow lines in the figure represent stages in the calculation. A straight line DE is drawn through the linear middle portion of the record. Extrapolation to zero time (i.e. the moment at which adenosine was added) gives the peak height F corresponding to the presence of 0.787 μmoles of adenosine. The time for 50% disappearance of adenosine can readily be determined by projection of the horizontals GH and IJ corresponding to 25% and 75% of the maximum peak height F: the time necessary to achieve a reduction (50%) in the peak height from J to H, and hence the time needed for inactivation of 0.394 μmoles of adenosine (50% of the 0.787 μmoles added) by the blood fraction in the incubate, corresponds to the time taken for the recorder pen to travel from J to H. (It is known that the reaction obeys Beer's law for adenosine concentrations between 20 and 100 μM [2], and it is assumed that the uptake of adenosine during the time interval between J and H proceeds at a constant velocity.) The uptake of adenosine is expressed in μmoles/hr/ml of blood fraction in the final incubate.

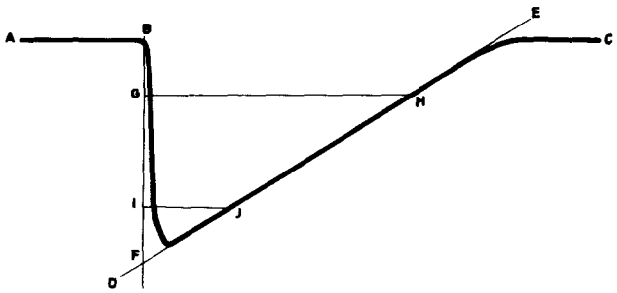

Fig. 1. Illustration of the technique used to calculate the rate of inactivation of adenosine by various blood fractions. For explanation, see Calculation Procedure in text.

4. Results and discussion

The results obtained using blood samples from 3 humans and 3 dogs are summarized in tables 1–3. One determination of the rate of inactivation of adenosine by total blood (intact or lysed) was made for each of the 6 samples; all other determinations were performed on aliquots drawn from a pool of equal volumes of the 3 human or canine blood samples.

4.1. *Total blood*

Table 2 shows that the rate of inactivation of adenosine by total human blood was about 10 times greater than that by canine blood. Our results are fully compatible with those reported for other studies on human [3,4] and canine [4,6] blood.

Table 1
Mean haematocrit and differential count for human and canine blood.

Parameter	Units	Human blood	Canine blood
Haematocrit	%	53.5	52
Erythrocytes	$10^9/cm^3$	4.4	6.1
Platelets	$10^8/cm^3$	2.75	3.30
Leucocytes	$10^6/cm^3$	6.6	9.7

Table 2
Rate of inactivation of adenosine by human and canine blood and blood fractions at pH 7.4 and 37°C.

Fraction	Rate of adenosine inactivation by stated fraction (μmoles/hr)				Ratio of lysed/intact blood	
	Intact blood		Lysed blood			
	Human	Canine	Human	Canine	Human	Canine
1 ml of total blood	10.5 ± 0.9	1.04 ± 0.16	11.4 ± 0.8	18.4 ± 1.6	1.09	17.7
10^9 erythrocytes	2.23	0.07	2.34	0.40	1.05	5.71
Erythrocytes/ml of total blood*	9.81	0.43	10.30	2.44		
10^8 platelets	0	0.39	0	4.87	–	12.5
Platelets/ml of total blood*	0	1.29	0	16.07		
1 ml of plasma	0.20	0.29	–	–	–	–
Plasma/ml of total blood	0.11	0.15	–	–		

* Calculated from the results given in table 1.

Table 3
Relative potencies of the various blood fractions as adenosine inactivators (total blood = 100).

Fraction	Intact blood		Lysed blood	
	Human	Canine	Human	Canine
Total blood	100	100	100	100
Erythrocytes	93.5	41.3	90.4	13.3
Platelets	0	124	0	87.3
Plasma	1.0	14.4	1.0	0.8

Calculated from the results given in table 2.

Haemolysis did not appreciably alter the rate of adenosine inactivation by total human blood (table 2). This finding is in agreement with those of Bunag et al. [3], but contrary to those of Koss et al. [4] who observed a 75% increase in activity on lysis. Haemolysis of canine blood caused a considerable increase in the rate of adenosine inactivation: approximately 18-fold in the present experiments, as compared with 8-fold in those of Koss et al. [4] and 10-fold in those of Kübler and Bretschneider [5]. The lower values reported for the earlier studies may have been due in part to incomplete lysis of the platelets (see below).

4.2. *Plasma*

The absolute level of adenosine deaminase activity in plasma was low for both species (table 2); as a percentage of the total (intact) blood activity it was about 1% in humans and about 14% in dogs (table 3). These results are in broad agreement with those of Koss et al. [5].

4.3. *Erythrocytes and platelets*

It is generally assumed that the erythrocytes are largely responsible for the uptake of adenosine from both human and canine blood [3–7]. However, table 3 shows that in the present study canine erythrocytes accounted for only a small part of the adenosine inactivation potency of the blood, and even this small part may be questioned in view of the difficulty of ridding the erythrocytes completely of platelets. Indeed, in a subsidiary experiment using canine erythrocytes sedimented 3 times in Ficoll, we did not observe any adenosine uptake by the cells or any adenosine deaminase activity on lysis.

The platelet fractions of canine blood, intact or lysed, showed much the greatest activity (table 3). The higher potency of the intact platelet fraction than of the total blood sample may have been due to adsorption from solution of some of the adenosine in the latter, perhaps onto the erythrocytes. By contrast, for human blood, the platelet fraction had negligible activity, and over 90% of the total potency resided in the erythrocyte fraction.

Further studies are in progress to investigate the differences in activity of the various blood fractions of other species.

Acknowledgements

The author is indebted to Dr. P.A.J.Janssen, Director of the Laboratory, for his continuous encouragement, and to Dr. A.E.F.Chandler for his help in the preparation of the manuscript.

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